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Editing Fusarium graminearum genome with CRISPR/Cas9

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Abstract

CRISPR/Cas9 technology can be used to mutate genomes and make a pathogenic organism less infectious. We used CRISPR/Cas9 plasmids constructed by a former undergraduate student to mutate three genes in the cereal fungal pathogen *Fusarium graminearum.* One of the genes (*AUR1*) is a visual marker and the other two genes (*MGV1* and *Tri5*) are essential for infection. The CRISPR/Cas9 plasmids also contain a hygromycin B resistance gene for selection. We transformed *F. graminearum* protoplasts and selected colonies on media containing hygromycin B. We then screened the target genes for mutations by PCR and gel electrophoresis. Unfortunately, we were unable to confirm mutations in the transformants. However, many fungi, including *F. graminearum*, are multinucleated and this made it difficult to isolate colonies with genetically identical nuclei (homokaryotes). Further work is needed to isolate homokaryotic cells.

Introduction

Fusarium graminearum is a pathogenic fungus that causes scab or *Fusarium* head blight disease in barley and wheat. The disease not only reduces yield, but it also contaminates the kernel with harmful toxins. Therefore, the disease causes economic loss and poses a health risk to humans and animals. Previous studies have shown that CRISPR/Cas9 can be used to introduce mutations in filamentous fungi (Nødvig *et al*., 2015). In this study, we used CRISPR/Cas9 to mutagenize three genes in *F. graminearum: AUR1* (a visual marker), *MGV1* (essential for sexual reproduction and infection), and *Tri5* (important for toxin production and infection). We hypothesize that silencing *MGV1* and *Tri5* could inhibit the ability of the fungus to infect barley.

Figure 1. Infected (left) vs. healthy barley (right).

Source: USDA

Figure 2: *F. graminearum growing* on artificial medium

Source: Pancaldi *et a-l*. 2010. Phytopathol Mediterr 49: 258-266.

Methods

Grow *F. graminearum*

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Isolate protoplasts

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Transform protoplasts with CRISPR/Cas9 plasmids

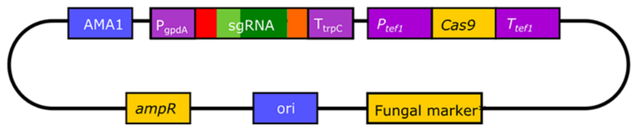

Figure 3. Basic plan of the CRISPR/Cas9 plasmids. The single guide RNA (sgRNA) is shown in light green.

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Grow protoplasts into fungal colonies

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Screen colonies using PCR

Results & Discussion

Figure 4. *F. graminearum* macrospores used to grow mycelia in liquid culture.

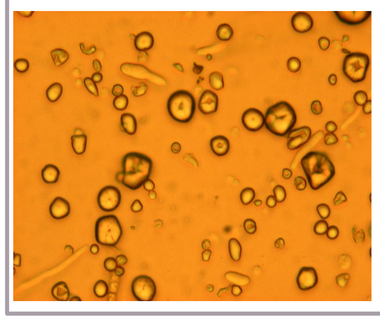

Figure 5. Protoplasts isolated from mycelia for transformation.

Acknowledgement

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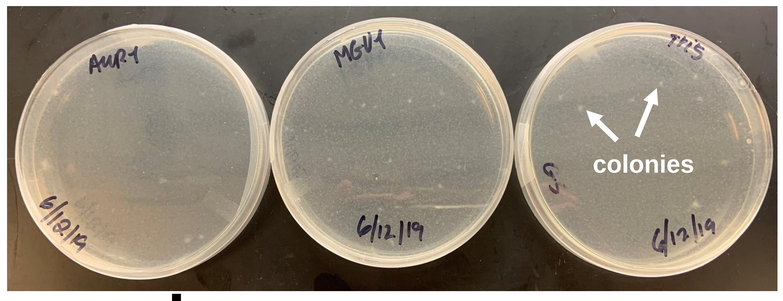
References

Akers R. 2019. Construction of CRISPR/Cas9 vectors for directed mutagenesis of *Fusarium graminearum*. *Honors Program Theses*. 368.

Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH. 2015. A CRISPR-Cas9 system for genetic engineering of filamentous fungi. PLoS ONE 10(7) 10.1371

Results & Discussion



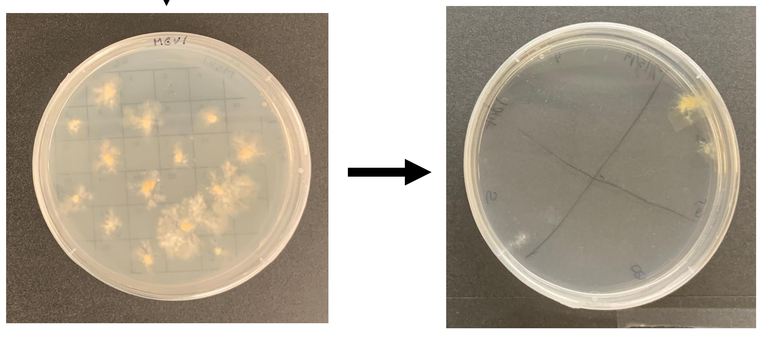


Figure 5. Selection of *F. graminearum* colonies.

Figure 6. Screening putative *F. graminearum* transformants by PCR. Only one band was detected using primers to amplify the target gene in the fungus and primers to amplify a segment of the plasmid DNA. Two bands would have indicated successful transformation.

Conclusions

Recovery of colonies on selective media suggests that they carry the CRISPR/Cas9 plasmids. However, we were unable to detect the plasmid DNA in these colonies by PCR. This might be due to the fact that *Fusarium graminearum* is multinucleated and not all are transformed. Further research is needed to tweak the screening process in order to isolate spores with a single nucleus, and to improve the transformation procedure.